

Supplementary Materials: Proteo-Transcriptomic Analyses Reveal a Large Expansion of Metalloprotease-Like Proteins in Atypical Venom Vesicles of the Wasp *Meteorus pulchricornis* (Braconidae)

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For Table S1, S3, S4, see excel files

Table S2. Genomes and Transcriptomes use in the study

	Species	Version	Number of CDS	Site	Reference (DOI)	NCBI Project
Genomes	<i>Aphidius ervi</i>	3.0	20,344	BIPAA	10.1186/s12864-020-6764-0	PRJNA3 47567
	<i>Apis mellifera</i>	HA3.1	23,471	NCBI	10.1186/s12864-019-5642-0	PRJNA4 71592
	<i>Campoletis sonorensis</i>	1.1	21,987	BIPAA	10.1186/s12915-020-00822-3	PRJNA5 90982
	<i>Cotesia congregata</i>	2.3	14,149	BIPAA	10.1038/s42003-020-01623-8	
	<i>Diachasma alloeum</i>	GCF_001 412515.1	19,692	NCBI	10.1093/gbe/evz 205.	PRJNA3 06876
	<i>Drosophila melanogaster</i>	r6.26	30,480	FLYBASE	10.1101/gr.1855 79.114	
	<i>Fopius arisanus</i>	ASM8063 6v1	18,906	NCBI	10.1534/g3.117.0 40741	PRJNA2 58104
	<i>Hyposoter didymator</i>	2.1	18,154	BIPAA	10.1186/s12915-020-00822-3	PRJNA5 89497
	<i>Lysiphlebus fabarum</i>	1.0	15,203	BIPAA	10.1186/s12864-020-6764-0	PRJNA5 87428
	<i>Microplitis demolitor</i>	GCF_000 572035.2	18,586	NCBI	10.1534/g3.118.2 00308	PRJNA2 51518
	<i>Nasonia vitripennis</i>	GCF_000 002325.3	24,846	NCBI	10.1126/science. 1178028	PRJNA1 3660
	<i>Venturia canescens</i>	1.1	23,401	BIPAA	10.1126/sciadv.1 501150	

Transcriptomes of venom glands	<i>Bracon nigricans</i>	1.0	57,686		10.1186/s12864-019-6396-4	PRJNA542369
	<i>Liragathis javana</i>	1.0	11,337	BIPAA		PRJNA734452
	<i>Meteorus pulchricornis</i>	1.0	16,828	BIPAA		PRJNA733444
	<i>Pimpla turionnalae</i>	1.0	347,311	https://zenodo.org/record/3545834#.YBgwJ3VKiV4	10.1186/s12864-019-6396-4	PRJNA555750
	<i>Psytalia concolor</i>	1.0	14,370		10.1038/srep35873	PRJNA262710
Transcriptome of antennae	<i>Meteorus pulchricornis</i>				10.1016/j.cbd.2017.01.002	PRJNA345399

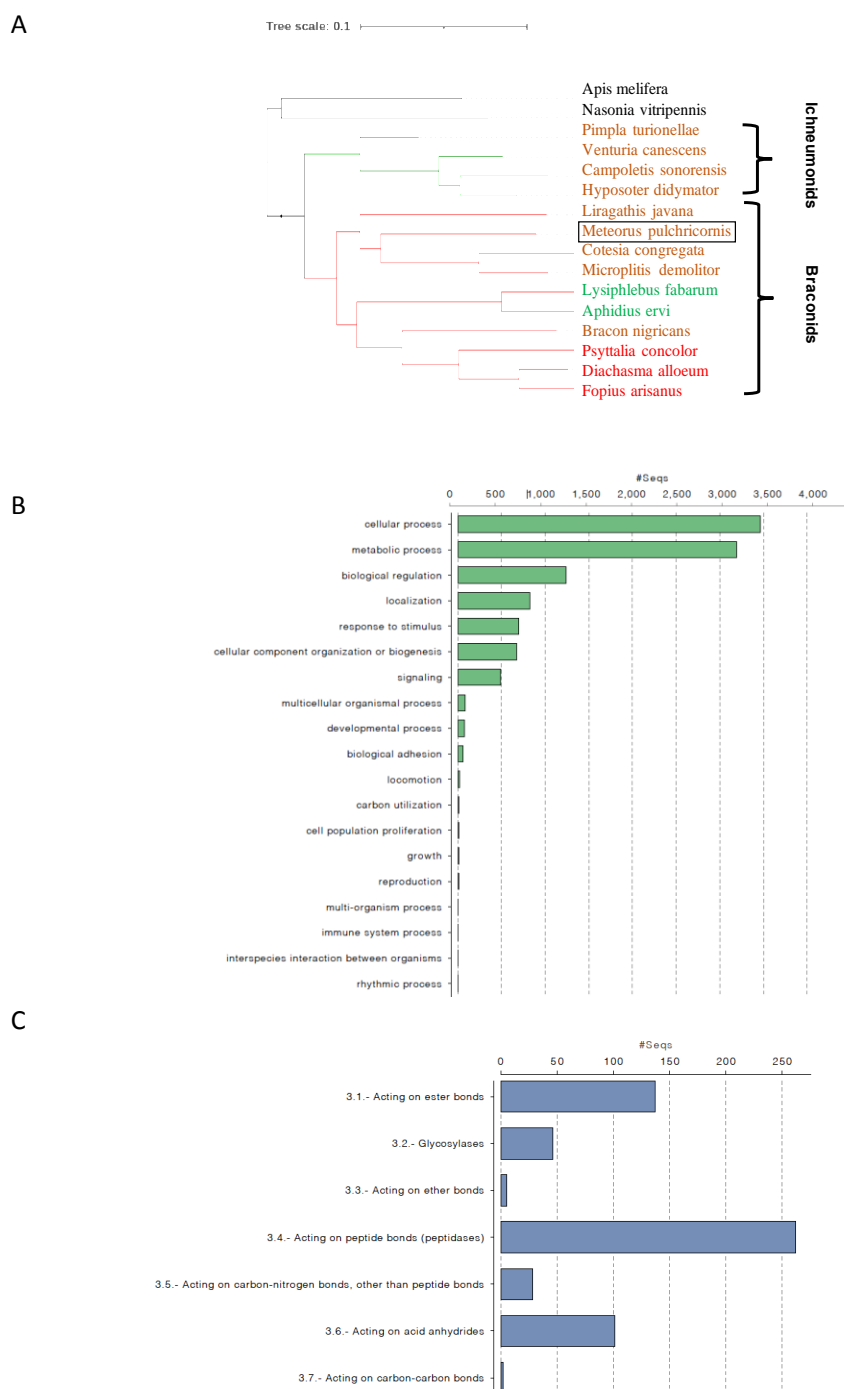


Figure S1. Phylogeny from Orthofinder and Blast2GO Short Reports. In A : phylogeny of the species from orthofinder (names: in black, non-Ichneumonidae; in orange, parasitoids of lepidopterans; in green, of aphids; in red, of dipterans). B, Second level GO categories of biological process and molecular function among functionally annotated *M. pulchricornis* transcripts. In C: Enzyme Code Level 3 Distribution.

MGPIIINFEVV **TFIMMYCEGI** **AG**EVINNDTI NLEYFQHPFF FNMVHAK**HQC**
 RKSCEDITMD DMTIQTDSI KSSCYGTND CRAMVADTV DLQYCRKNNS
 TDIVWAFDSQ RKVEYGQKSN CGDGATEYTV PRVGYSGGSG CPICVCTCYE
 ATSGMASNKG KWWFSAVPQT SDENKVIITGV KFVEINHTIF LQIKQNPFI
 DTTGNWKTLD EEVEAEEGVS KDTRLLLTDK RRSMLLDEFE LPDGYVITGV
 KFEEATIPTT SLLAIRLSVR STKLTSKDGL SESETSTWII GTTSKLKSTH
 LKWLPSMGL NPDILKTHMF GIGDLKDFG KLEPTISAE PCEATILKET
PMGGLQLLV NISEKQKIYP **R**VAPLGIDPD PESKEEEDDD DDMGDNGDSA
 STTNLQFILI ALVPLSIIFN LFD

MEKLSMNLVV **ILMIHCKEI** **AG**DLINLKYF **REAIIFSEHD** **LGTCGFGCGK**
 LTLSETANYK STFTKQVCDG YLVNCQITVG KANASLQFCL DDQNSRMVWA
 ENKQYGAQY GIQPREICITP INEPLKTKDS ETCEACVCTC YHERESSEDE
 NVWRFLAE TSGDAKVVVG VRFTLIDNII HLQIKENDLI STTNGTWKSI
 TVDSDRDGD TRKYATLSHN LQTIKLNQIF SPENSIVITGV KEVKVPNLDK
 KGFALELQIR STKLNDEDA LDPHQDWHG GDSPTLTAGP RWWPDRGIHN
 TPLPIVSLNR TSLNKDFGMT **TNPQFYADPV** **VSCAQHDYPL** **KGIQLLVEL**
GGQEVIIPR I SPAIKGSTL PDYLDYESNS EPELTDSANT ANLQFCLIAM
 LPLLTIWNL N

MGPIIINFEVV **TFIMMYCEGI** **AG**EVINNDTI NLEYFQHPFF FNMVHAK**HQC**
 RKSCEDITMD DMTIQTDSI KSSCYGTND CRAMVADTV DLQYCRKNNS
 TDIVWAFDSQ RKVEYGQKSN CGDGATEYTV PRVGYSGGSG CPICVCTCYE
 ATSGMASNKG KWWFSAVPQT SDENKVIITGV KFVEINHTIF LQIKQNPFI
 DTTGNWKTLD EEVEAEEGVS KDTRLLLTDK RRSMLLDEFE LPDGYVITGV
 KFEEATIPTT SLLAIRLSVR STKLTSKDGL SESETSTWII GTTSKLKSTH
 LKWLPSMGL NPDILKTHMF GIGDLKDFG KLEPTISAE PCEATILKET
PMGGLQLLV NISEKQKIYP **R**VAPLGIDPD PESKEEEDDD DDMGDNGDSA
 STTNLQFILI ALVPLSIIFN LFD

MEKLSMNLVV **ILMIHCKEI** **AG**DLINLKYF **REAIIFSEHD** **LGTCGFGCGK**
 LTLSETANYK STFTKQVCDG YLVNCQITVG KANASLQFCL DDQNSRMVWA
 ENKQYGAQY GIQPREICITP INEPLKTKDS ETCEACVCTC YHERESSEDE
 NVWRFLAE TSGDAKVVVG VRFTLIDNII HLQIKENDLI STTNGTWKSI
 TVDSDRDGD TRKYATLSHN LQTIKLNQIF SPENSIVITGV KEVKVPNLDK
 KGFALELQIR STKLNDEDA LDPHQDWHG GDSPTLTAGP RWWPDRGIHN
 TPLPIVSLNR TSLNKDFGMT **TNPQFYADPV** **VSCAQHDYPL** **KGIQLLVEL**
GGQEVIIPR I SPAIKGSTL PDYLDYESNS EPELTDSANT ANLQFCLIAM
 LPLLTIWNL N

Score	Expect	Method	Identities	Positives	Gaps
190 bits(482)	2e-60	Compositional matrix adjust.	133/427(31%)	207/427(48%)	20/427(4%)
Query 1		MGPIIINFEVTFIMMYCEGIAGEVINNDTINLEYFQHPFFNNMVHAKHQC			60
Sbjct 1		MEKLSMNLVILMIHCKEIAG-----DLINLKYFREAIIFSE-HDLGTCGFGCGKLTLS			54
Query 61		DMTIQTDSIKSSCYGTNDCRAMVADTVDLQYCRKNNSDIVWAFDSQ-VEYG-QK			118
Sbjct 55		ETANYKSTFTKQVCDGYLVNCQITVGKANASLQFCLDDQNSRMVWAENKQYGAQYGIQ			114
Query 119		SNCGDGAETTVPRVGYSGGSGCPICVCTCYEATSGMASNKGKWWFSAVPQSDENKVI			178
Sbjct 115		RECITPINE----PLKTKDSECEACVCTCYHERES-SEDEVWRFLAEETSGDAKVV			169
Query 179		GVKFVEINHTIFLQIKQNPFIIDTTGNWKTLDVEEAEEGVSQDTR--LLTDRRSLML			236
Sbjct 170		GVFTLIDNIIHLQIKENDLISTNGTWKSIITVDSDRD---GPDTRKYATLSHNQTIKL			226
Query 237		DEFELPDGYVITGVKFEEATIPTTSLAIRLSVRSTKLTSKDGLSESETSTWII GTTSKL			296
Sbjct 227		NQIFSPENSIVITGVKFVKVPNLDKKGFALELQIRSTKLNDEDA LDPHQDWHGDSPTL			286
Query 297		KSTHLKWLPSMGLNPDILKTHMFGIGDLKDFGKLEPTISAEPCATILKETPMGGLQ			356
Sbjct 287		TAGP-RWWPDRGIHNTPLFI-VSLNRSTLNDKDFGMTTNPQFYADPVVSCAQHDYPLKGIQ			344
Query 357		LLLVNISEKQKIYPRVAPLGIDPD PESKEEEDDDDMGDNGDSASTTNLQFILI ALVPLS			416
Sbjct 345		LLLVGGQEVIIIPRISPAIKGSTLPDYLDYESNEPELTDSANTANLQFCLIAMLPL			404
Query 417		IIFNLFD 423			
Sbjct 405		TINWNLN 411			

Figure S2. Comparison of Contig 38.p1 and .p2. Highlight in yellow putative signal peptide. In red peptides found by mass spectrometry in the total venom or the MpVLPs samples (in brown those found only in TV). The lower figure shows the Blastp comparison of the two proteins.



Figure S3. CLUSTAL multiple sequence alignment of the *M. pulchricornis* venom Metalloprotease. Proteins with name of the same color clustered together and may form a clade as shown by the clustal clustering on the left. AA in blue, putative Furin site; AA in red highlight in cyan, putative triad involved in Ca²⁺ binding; Conserved Cys in red highlight in yellow; Highlight in grey sequence

features, AA in brown canonic Zn²⁺ binding/active site and in purple Met-turn.

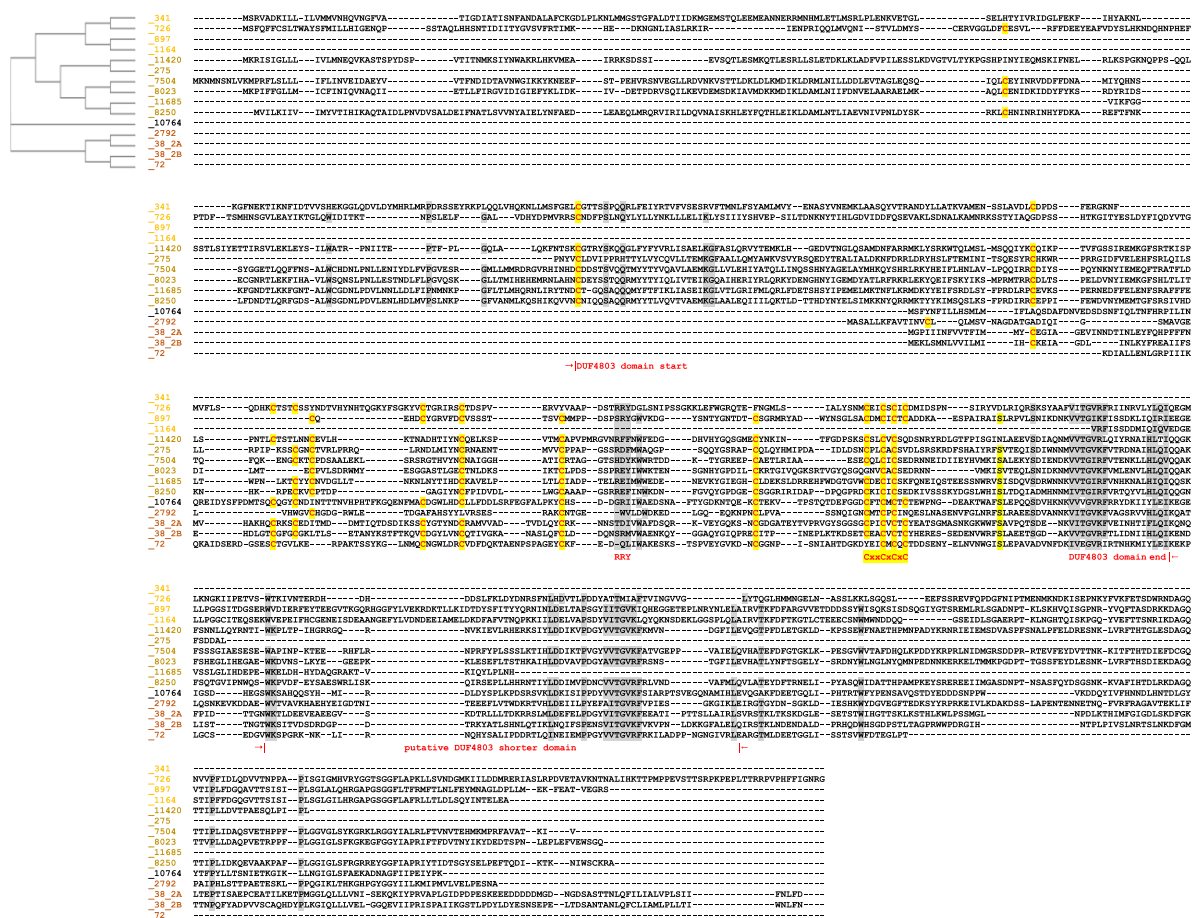


Figure S4. CLUSTAL multiple sequence alignment of the DUF4803 containing proteins. Proteins with name of the same color clustered together and may form a clade as shown by the clustal clustering on the left. Conserved Cys in red highlight in yellow; Highlight in grey sequence features and highly conserved AA.